Appendix 2

This appendix presents derivation of the analytical expressions for relationship between the critical distances and the ratio of prevalences, and describes how the critical distances can be approximated over ratios and topographies of standard deviations as a function of the ratio of prevalences for each of the two topographies of prevalences, $TPrevA$ and $TPrevB$.

Topography of prevalences $A$ ($TPrevA$)

Expressing critical distances as a function of critical percentages

To examine the relationship between the critical partitioning and non-partitioning distances, or $DCritP$ and $DCritNP$, and the ratio of prevalences, or $F_r$, we will first consider the topography of prevalences $A$, or $TPrevA$. As discussed in the text, one has to account for two different topographies of standard deviations for each topography of prevalences. Thereby two combinations of topographies, $TPrevA$, $TStDevA$ (Fig. 2 A) and $TPrevA$, $TStDevB$ (Fig. 2 B), need to be considered separately.

In Appendix 1 we derived an expression (left-hand side of Appendix 1 Eq. 3) for the distance between reference limits ($D$) as a function of the ratio between standard deviations ($R$), assuming equal prevalences. These calculations concerned $TStDevB$. In a similar way, one can easily obtain the following expression for $D$ in the case of $TStDevA$, assuming equal prevalences:

$$D = Q(p_a;0;1) - Q(p_b;0;R) + (R-1)*Q(0.025;0;1) \quad \text{(Appendix 2 Eq. 1)}$$

If $F_r > 1$, the proportions $p_a$ and $p_b$ in Appendix 2 Eq. 1 need to be adjusted for $F_r$. Turning back to our
example with $F_r = 2.0$ in the section “Proportions of two gaussian distributions, having unequal prevalences, outside the reference limits of the combined distribution” of the text, we had $p_a = 0.025 + 2 \times x$ and $p_b = 0.025 - x$. Generalization of these equations leads to $p_a = 0.025 + F_r \times x$ and $p_b = 0.025 - x$. Solving $x$ from the equation for $p_a$ and inserting this $x$ into the equation for $p_b$, we obtain the following relationship between $p_a$ and $p_b$, valid for $TPrevA$:

$$p_b = 0.025 - \frac{p_a - 0.025}{F_r}$$  \hspace{1cm} (Appendix 2 Eq. 2)

Observe that when $F_r = 1.0$, Appendix 2 Eq. 2 is reduced to Eq. 2. Inserting $p_b$ from Appendix 2 Eq. 2 into Appendix 2 Eq. 1 gives the general expression for the distance between reference limits $D_{AA}$ as a function of the proportion variable $p_a$ with $R$ and $F_r$ as parameters, valid for the topographic combination $TPrevA$, $TStDevA$ (the index “AA” of $D$ is referring to this topographic combination to distinguish it from later expressions for $D$, referring to other combinations of topographies):

$$D_{AA} = Q(p_a;0;1) - Q(0.025 -(p_a-0.025)/F_r;0;R) + (R-1)Q(0.025;0;1)$$  \hspace{1cm} (Appendix 2 Eq. 3)

Although this equation offers an accurate relationship between critical distances and proportions for each combination of the parameters $R$ and $F_r$, it and later similar equations for other topographies may be tedious to use in practical work. Hence, as an alternative to the use of these equations as a means to account for the prevalence effect, we will construct a generally valid nomogram showing plots of the critical distances vs. $F_r$, to make this accounting easy in practice. This nomogram will present approximate critical partitioning and non-partitioning distances for each $F_r$, calculated as averages of the critical distances over $R = 1.0$ and $R = 1.36$ and over the two topographies of standard deviations for each topography of prevalences.

**Determining a maximum value for $F_r$**

Wishing to illustrate the relationship between critical distances and $F_r$, we will first have to decide on an
appropriate range for $F_r$. In principle, $F_r$ does not have a maximum value, but we will determine a range for it by limiting the precision of the critical distances to two decimals, because higher precision than that is in practice not needed for correlating critical distances to critical proportions. In order to determine this kind of maximum value for $F_r$, one has to consider the actual expressions for the critical partitioning distance, or $DCritP_{AA}$, and the critical non-partitioning distance, or $DCritNP_{AA}$, both corresponding to the topographic combination $TPrevA$, $TStDevA$, as a function of $F_r$. These can be obtained by inserting the critical partitioning proportion, or $p_a = pCritP = 0.041$, and the critical non-partitioning proportion, or $p_a = pCritNP = 0.032$, respectively in Appendix 2 Eq. 3, and averaging over $R = 1.0$ and $R = 1.36$:

$$DCritP_{AA} = Q(0.041;0;1) – 1.18*Q(0.025-0.016/F_r;0;1) + 0.18*Q(0.025;0;1)$$ (Appendix 2 Eq. 4)

$$DCritNP_{AA} = Q(0.032;0;1) – 1.18*Q(0.025-0.007/F_r;0;1) + 0.18*Q(0.025;0;1)$$ (Appendix 2 Eq. 5)

Asymptotes for these critical distances can be calculated by omitting the terms having $F_r$ as divisor in the arguments of the quantile functions including this divisor, because as $F_r$ is increased toward infinity, these terms approach zero. In this way, we obtain the asymptotic values of 0.22 s and 0.11 s for $DCritP_{AA}$ and $DCritNP_{AA}$, respectively, averaged over $R = 1.0$ and $R = 1.36$. Numerical calculations will show further that these asymptotic distances are reached at $F_r = 76.6$ and $F_r = 19.8$, respectively, i.e. both asymptotic distances are reached by $F_r = 76.6$. Hence, $F_r = 76.6$ is an approximate maximum value for $F_r$, covering the topographic combination $TPrevA$, $TStDevA$. Just to illustrate an extreme case like this, Appendix 2 Fig. 1 A shows the lower ends of two gaussian distributions with $F_r = f_b/f_a = 76.6$ and $R = 1.0$. Because distribution $b$ is extremely “heavy” as compared to distribution $a$, distribution $a$ would hardly be visible if both distributions were plotted using the same frequency scale. Distribution $a$ is therefore presented as multiplied by $F_r$, so that both distributions have the same size.

Appendix 2 Figure 1
Because the lower common reference limit is in practice identical with the lower reference limit of distribution \( b \), the proportion \( p_b \) is approximately 2.5% – in accordance with Appendix 2 Eq. 2 – and the proportion \( p_a \) is determined at \(-1.96 s + 0.22 s = -1.74 s\). This proportion is in fact 4.1%, i.e. the critical partitioning proportion. It is obvious that 0.22 \( s \) is indeed a minimum for \( DCritP \), assuming \( TPrevA \), \( TSdVA \), Appendix 2, Note 2 because if \( D \) were shorter, \( p_a \) could not reach the critical value of 4.1%.

Compare the situation in Appendix 2 Fig. 1 A to that in Appendix 2 Fig. 1 B, illustrating the same distributions that have equal prevalences this time. At the critical distance, corresponding to \( p_a = 4.1\% \) and \( p_b = 0.9\% \) (Eq. 2), the lower common reference limit must lie at the same position as in Fig. A, or at \(-1.74 s\) from the mean of distribution \( a \) because otherwise \( p_a \) could not be equal to 4.1%. But instead of coinciding with the lower common reference limit, the lower reference limit of distribution \( b \) lies this time at a distance of 0.41 \( s \) to the right from the common limit, resulting in \( DCritP = 0.63 s \). That this is indeed the case, can be verified by inserting \( p_a = 4.1\% \), \( Fr = 1.0 \), and \( R = 1.0 \) into Appendix 2 Eq. 3.

_Averaging critical distances over ratios of standard deviations_

Knowing the range of \( Fr \), we will next plot \( D_{AA} \) vs. \( p_a \) for various \( Fr \) at \( R = 1.0 \) and \( R = 1.36 \), using Appendix 2 Eq. 3. Such pairs of curves for each of \( Fr = 1.0, 2.0, 5.0, \) and 76.6 are shown in Appendix 2 Fig. 2. Notice that the dispersion of the pairs of curves is decreased as \( Fr \) is increased in the way that finally, for \( Fr = 76.6 \), the curves at \( R = 1.0 \) and \( R = 1.36 \) are not visually separable from each other (cf. Appendix 2 Note 2). However, this finding is not valid for all of the four combinations of topographies, depicted in Fig. 2. Some of the critical distances as averaged over \( R = 1.0 \) and \( R = 1.36 \) at the critical proportions (\( pCritNP = 3.2\% \) and \( pCritP = 4.1\% \)), that are calculable using Appendix 2 Equations 4-5 and that also can be read from Table 1 A, are indicated using arrows. Observe that the critical partitioning distance corresponding to \( Fr = 76.6 \) (0.22 \( s \)) is shorter than the critical non-partitioning distance corresponding to \( Fr = 1.0 \) (0.27 \( s \)).
Appendix 2 Figure 2

In the Appendix of our previous paper (5) we showed that the critical distances lay close to each other for the two topographies of standard deviation, because the dispersion of the $p_a$ vs. $D$ curves as plotted for $R = 1.0$ and $R = 1.5$ was rather modest and similar at the critical proportions for both $TStDevA$ and $TStDevB$. But because this observation was made for the particular case of $F_r = 1.0$, we must apparently examine the behaviour of the critical distances for both $TStDevA$ and $TStDevB$ as a function of $F_r$, if we wish to generalise our finding concerning the applicability of the same approximate distance criteria for both of them. If the critical distances as averaged over $R = 1.0$ and $R = 1.36$ (observe that instead of $R = 1.5$, we will in this study use the accurate value of $R = 1.36$, calculated in Appendix 1, because this value leads to smaller dispersion and better quality of the approximate critical distances) for both topographies of standard deviations, $TStDevA$ and $TStDevB$, lie reasonably close to each other throughout the range of $F_r$, these distances as averaged further over $TStDevA$ and $TStDevB$ should be useful as final approximate critical distances. We will next show that this is in fact the case, but before that, we will derive the expressions corresponding to Appendix 2 Equations 3-5 for the topographic combination $TPrevA$, $TStDevB$.

The expression for $D_{AB}$, or the distance between reference limits for $TPrevA$, $TStDevB$, is as follows (cf. Appendix 2 Eq. 3 and Appendix 1 Eq. 3):

$$D_{AB} = Q(p_a;0;R) - Q(0.025-(p_a-0.025)/F_r;0;1) - (R-1)*Q(0.025;0;1) \quad \text{(Appendix 2 Eq. 6)}$$

By averaging Appendix 2 Eq. 6 over $R = 1.0$ and $R = 1.36$, we will obtain the following expressions for $DCritP_{AB}$ and $DCritNP_{AB}$ as functions of $F_r$:

$$DCritP_{AB} = 1.18*Q(0.041;0;1) - Q(0.025-0.016/F_r;0;1) - 0.18*Q(0.025;0;1) \quad \text{(Appendix 2 Eq. 7)}$$
\[ DCritNP_{AB} = 1.18*Q(0.032;0;1) - Q(0.025-0.007/F_r;0;1) - 0.18*Q(0.025;0;1) \quad (\text{Appendix 2 Eq. 8}) \]

The asymptotes of \( DCritP_{AB} \) and \( DCritNP_{AB} \) are 0.26 \( s \) and 0.13 \( s \), respectively, as can easily be obtained from Appendix 2 Equations 7-8. These values are well reached by \( F_r = 76.6 \) which was the maximum value of \( F_r \) for \( TStDevA \) (to be precise, \( DCritP_{AB} \) reaches its asymptote at \( F_r = 61.2 \) and \( DCritNP_{AB} \) at \( F_r = 15.5 \)). Hence, we do not have to expand the range of \( F_r \) beyond 76.6 in order to have the critical distances under control with a precision of two decimals for either topography of standard deviations, assuming \( TPrevA \).

**Averaging critical distances over topographies of standard deviation**

For \( F_r = 1.0 \) we observed earlier (5) that the dispersion of the \( p_a \) vs. \( D \) curves for various values of \( R \) was slightly smaller for \( TStDevB \) than for \( TStDevA \), assuming equal prevalences. However, the ratio of the dispersions varies between these two topographies for \( F_r > 1.0 \). As was stated above, the dispersion of the \( D_{AA} \) vs. \( p_a \) curves decreases as \( F_r \) is increased (Appendix 2 Fig. 2). Instead, the dispersion of \( D_{AB} \) vs. \( p_a \) curves remains constant throughout the range of \( F_r \) because the term of Appendix 2 Eq. 6 including \( F_r \) is eliminated as the curve at \( R = 1.0 \) is subtracted from the curve at \( R = 1.36 \). The dispersion of the \( D_{AA} \) vs. \( p_a \) curves exceeds in fact that of the \( D_{AB} \) vs. \( p_a \) curves only for the range \( F_r \leq 1.5 \). In order to compare the averages of the critical distances over \( R = 1.0 \) and \( R = 1.36 \) between the two topographies of standard deviation as a function of \( F_r \), we simply calculate the difference between Appendix 2 Eq. 4 and 7 for the critical partitioning distances, and the difference between Appendix 2 Eq. 5 and 8 for the critical non-partitioning distances:

\[ DCritP_{AA} - DCritP_{AB} = 0.18*(2*Q(0.025;0;1) - Q(0.041;0;1) - Q(0.025-0.016/F_r;0;1)) \quad (\text{Appendix 2 Eq. 9}) \]

\[ DCritNP_{AA} - DCritNP_{AB} = 0.18*(2*Q(0.025;0;1) - Q(0.032;0;1) - Q(0.025-0.007/F_r;0;1)) \quad (\text{Appendix 2 Eq. 10}) \]
Because both differences given by Appendix 2 Equations 9-10 are monotonically decreasing functions of \( F_r \), Appendix 2, Note 3 there will be no other extreme values for these differences except those obtained at the end points of the range of \( F_r \), or \( F_r = 1.0 \) and \( F_r = 76.6 \). Inserting these values into Appendix 2 Equations 9-10, we obtain 0.03 \( s \) as the maximum value and –0.04 \( s \) as the minimum value for the difference of the critical partitioning distances, and 0.01 \( s \) and –0.02 \( s \), respectively, for the difference of the critical non-partitioning distances. The situation for the critical partitioning distances is illustrated in Appendix 2 Fig. 3.

**Appendix 2 Figure 3**

In Appendix 2 Fig. 3, the uppermost and the lowermost curve of the three curves corresponding to \( F_r = 1.0 \) are the same as the two uppermost curves in Appendix 2 Fig. 2 (observe the changed scale, however), i.e. the \( D_{AA} \) vs. \( p_a \) curves at \( R = 1.36 \) and \( R = 1.0 \), respectively. The middle and the lowermost curve are these curves for \( TStDevB \). Hence, \( DCritP_{AA} \) and \( DCritP_{AB} \) are identified as the midpoints, indicated using arrows, of these two pairs of curves, respectively. The results of this graphical presentation agree well with the calculated values of Table 1 A. The lower curve in the lower part of Appendix 2 Fig. 3, corresponding to \( F_r = 76.6 \), represents three curves simultaneously: the two coinciding \( D_{AA} \) vs. \( p_a \) curves at \( R = 1.36 \) and \( R = 1.0 \), shown as the lowermost curve in Appendix 2 Fig. 2, and the \( D_{AB} \) vs. \( p_a \) curve at \( R = 1.0 \). The upper curve corresponding to \( F_r = 76.6 \) in Appendix 2 Fig. 3 is the \( D_{AB} \) vs. \( p_a \) curve at \( R = 1.36 \). Observe that the critical distances for the two topographies of standard deviation have reversed orders for \( F_r = 1.0 \) and \( F_r = 76.6 \), as was discussed above, and that these distances lie in fact reasonably close to each other throughout the range of \( F_r \).

As a conclusion, the critical distances corresponding to the two topographies of standard deviations lie at most 0.04 \( s \) from each other at any value of \( F_r \), assuming \( TPrevA \) and \( R < 1.36 \). Hence, averages of them should be appropriate as overall approximate critical distances, applicable to both topographies of standard deviations. We will accordingly present the averages of the critical partitioning and non-partitioning
distances from Appendix 2 Equations 4 and 7, and from Appendix 2 Equations 5 and 8, respectively, as our suggestions for approximate critical distances at each $F_r$. However, in order to determine an appropriate range of $F_r$ for graphical presentation of the critical distance vs. $F_r$ curves, we will consider this range also from the practical point of view.

In practice, $F_r$-values as high as 76.6 must be quite unusual in reference interval studies because $F_r = 76.6$ would correspond to prevalences 0.987 and 0.013. Moreover, critical distances as calculated using Appendix 2 Equations 4-5 and 7-8 change very little beyond $F_r = 10.0$. In fact, at $F_r = 10.0$ $DCritP_{AA} = 0.25$ $s$, $DCritNP_{AA} = 0.12$ $s$, $DCritP_{AB} = 0.29$ $s$, and $DCritNP_{AB} = 0.14$ $s$, which lie quite close to (at most 0.03 $s$) from the respective asymptotic values for these critical distances, calculated earlier and listed lowermost in Table 1 A. It seems therefore feasible to apply the averages of the critical distances over $F_r = 10.0$ and $F_r = 76.6$, whenever $F_r > 10.0$. These averages are $DCritP_{ave} = 0.26$ $s$ and $DCritNP_{ave} = 0.13$ $s$ (we will denote with “ave” the averages of the critical distances over both topographies of standard deviations, but in this case the “ave”-values are additionally averaged over the two points of $F_r$, $F_r = 10.0$ and $F_r = 76.6$). We will accordingly restrict the nomogram (Fig. 3) illustrating the relationship between critical distances and $F_r$ to the range $1 \leq F_r \leq 10$, to improve the resolution and thereby the usefulness of this nomogram. Fig. 3 shows a plot of $DCritP_{ave}$ and $DCritNP_{ave}$ as a function of $F_r$, lying within this range, as calculated from Appendix 2 Equations 4-5 and 7-8. The critical distances at selected values of $F_r$ are listed in Table 1 A.

Until now, we have considered only $p_a$, or the larger one of the proportions outside the common reference limit (Fig. 1 A), composing the nomogram in Fig. 3 for $TPrevA$ to make it correspond to the critical values of 4.1% and 3.2% for $p_a$. However, because the sum of $p_a$ and $p_b$ is not a constant but a function of $F_r$ (Appendix 2 Eq. 2), the behaviour of $p_b$ is a separate concern whenever $F_r > 1.0$. But when the critical values of 4.1% and 3.2% for $p_a$ are inserted into Appendix 2 Eq. 2, we will obtain $p_b > 0.9\%$ and $p_b > 1.8\%$, respectively, for $F_r > 1.0$. Hence, $p_b$ lies safely above its critical values for the critical values of $p_a$, assuming $TPrevA$. 
Topography of prevalences B (TPrevB)

To obtain the relationship between $p_a$ and $p_b$ for TPrevB, we simply substitute the inverse of $F_r$ for $F_r$ in Appendix 2 Eq. 2:

$$p_b = 0.025 - F_r^* (p_a - 0.025) \quad \text{(Appendix 2 Eq. 11)}$$

If $p_a = 4.1\%$, $p_b$ calculated from Appendix 2 Eq. 11 lies below 0.9\% for any $F_r > 1.0$, and values of $p_a$ exceeding 4.1\% result in still smaller values of $p_b$. On the other hand, $p_a$ cannot reach its critical value as long as the values of $p_b$ lie in its allowed range. Consequently, instead of controlling $p_a$ against larger values than the critical ones, as we did for TPrevA, we must for TPrevB control $p_b$ against smaller values than its critical ones. Solving $p_a$ from Appendix 2 Eq. 11 and expressing the distances in Appendix 2 Equations 3 and 6 as a function of $p_b$ gives the analogous general expressions for the distances $D_{BA}$ and $D_{BB}$, corresponding to TPrevB, TStDevA and TPrevB, TstDevB, respectively:

$$D_{BA} = Q(0.025 + (0.025 - p_b)/F_r; 0; 1) - Q(p_b; 0; R) + (R-1)*Q(0.025; 0; 1) \quad \text{(Appendix 2 Eq. 12)}$$

$$D_{BB} = Q(0.025 + (0.025 - p_b)/F_r; 0; R) - Q(p_b; 0; 1) - (R-1)*Q(0.025; 0; 1) \quad \text{(Appendix 2 Eq. 13)}$$

Inserting $p_b = 0.9\%$ and $p_b = 1.8\%$ into Appendix 2 Equations 12-13, and averaging these distances over $R = 1.0$ and $R = 1.36$, we will obtain the following expressions required to calculate the approximate critical distances as a function of $F_r$ for both topographies of standard deviation and corresponding to TPrevB:

$$DCrit_{PBA} = Q(0.025 + 0.016/F_r; 0; 1) - 1.18*Q(0.009; 0; 1) + 0.18*Q(0.025; 0; 1) \quad \text{(Appendix 2 Eq. 14)}$$

$$DCrit_{NPBA} = Q(0.025 + 0.007/F_r; 0; 1) - 1.18*Q(0.018; 0; 1) + 0.18*Q(0.025; 0; 1) \quad \text{(Appendix 2 Eq. 15)}$$
\[ DCritP_{BB} = 1.18 \times Q(0.025 + 0.016/F_r;0;1) - Q(0.009;0;1) - 0.18 \times Q(0.025;0;1) \quad \text{(Appendix 2 Eq. 16)} \]

\[ DCritNP_{BB} = 1.18 \times Q(0.025 + 0.007/F_r;0;1) - Q(0.018;0;1) - 0.18 \times Q(0.025;0;1) \quad \text{(Appendix 2 Eq. 17)} \]

The asymptotes of \( DCritP_{BA} \), \( DCritNP_{BA} \), \( DCritP_{BB} \), and \( DCritNP_{BB} \) are 0.48 s, 0.16 s, 0.41 s, and 0.14 s, respectively, and using similar reasoning as we used for \( TPrevA \), we can find out that the meaningful range of \( F_r \) extends up to 43.1 for \( TPrevB \). Further averages of the critical distances over the two topographies of standard deviation \( DCritP_{Bave} \) and \( DCritNP_{Bave} \) are plotted as functions of \( F_r \) in Fig. 3. The range of \( F_r \) in Fig. 3 is \( 1 \leq F_r \leq 10 \) also for \( TPrevB \), but the values \( DCritP_{Bave} = 0.46 \) s and \( DCritNP_{Bave} = 0.16 \) s can be used as reasonable compromise values whenever \( F_r > 10.0 \) (cf. Table 1 B and the discussion above for \( TPrevA \)). Critical distances at selected values of \( F_r \) for \( TPrevB \) are presented in Table 1 B.

Difference of the critical partitioning distances, or \( DCritP_{BA} - DCritP_{BB} \), analogous to the difference \( DCritP_{AA} - DCritP_{AB} \) of Appendix 2 Eq. 9 but corresponding to \( TPrevB \) (for brevity, we will omit presenting the expressions corresponding to Appendix 2 Equations 9-10 in the case of \( TPrevB \)), varies between 0.03 and 0.07 as a function of \( F_r \), and that of the critical non-partitioning distances, or \( DCritNP_{BA} - DCritNP_{BB} \), varies between 0.01 and 0.02. The difference of the critical partitioning distances thus varies slightly more in absolute terms for \( TPrevB \) than for \( TPrevA \). However, because these differences will be divided by two to account for the averaging over the two topographies of standard deviations, the quality of the final approximate critical distances seems to be reasonably good for both topographies of prevalences throughout the range of \( F_r \).

As Fig. 3 and Tables 1 A and B show, the shrinkage of the critical distances as functions of \( F_r \) is less dramatic for \( TPrevB \) than for \( TPrevA \). Because the curves for the partitioning distances between these two topographies differ considerably from each other, the averaging process that we have been pursuing in order to develop generally applicable approximate critical distances, cannot be extended to the topographies of prevalences. One must therefore always observe these topographies, even when applying
the approximate distance criteria.